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APPENDIX B

NASA INTERIM REQUIREMENTS FOR BIOCLEAN FACILITIES

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HEADQUARTERS

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

WASHINGTON, D. C.

INTERIM REQUIREMENTS FOR BIOCLEAN FACILITIES

1. SCOPE

1.1 Purpose: This requirement is for the design and operation of bioclean facilities for the manufacture, assembly, testing, and launch of spacecraft required by NASA to be decontaminated or sterilized before flight.

1.2 Authority: The authority for this requirement is to be found in NASA Management Manual 4-4-1, dated March 1963, and in subsequent revisions and additions to that document.

1.3 Scope: This document establishes the specific requirements that must be imposed on the design and operation of clean rooms and clean work stations to reduce microbiological contamination to a level required by the procedures for reliable spacecraft decontamination or sterilization.

1.4 Basis: Basic clean room and clean work station specifications shall be used as the basis for this requirement. Any one, or a combination of more than one, of the following specifications or their revisions shall be used as specified by the NASA Project or Center and concurred in by the Bioscience Programs Office:

> Federal Standard No. 209, December 16, 1963. Clean Room and Work Station Requirements, Controlled Environment.
>
> U. S. Air Force Technical Order 00-25-203, Standards and Guidelines for the Design and Operation of Clean Rooms and Clean Work Stations, July, 1963.
>
> Marshall Space Flight Center, Standard 246, July 29, 1963. Standard for Design and Operational Criteria of Controlled Environmental Areas.

In the event of conflict between this document and the above specification(s) used as a basis, this requirement shall take precedence.

1.5 Level: The level of physical cleanliness required under the specifications above shall be specified by the NASA Project or Center with approval by the Bioscience Programs Office. The following requirements for levels of microbiological contamination of a clean space or work area will apply to any and all levels of physical cleanliness that may be specified.

2. REQUIREMENTS - BIOCLEAN ROOMS

The bioclean room shall be operated in such a manner that it will meet or exceed the following requirements. Failure to meet any single standard at any time shall be cause for immediate measures to correct the situation. Continued failure to meet the standards could result in failure to obtain certification for flight.

2.1 MICROBIOLOGICAL

2.1.1 Ventilation Inlet Air: The concentration of viable particles in the ventilation inlet air shall not exceed an average of one (1) viable particle per cubic foot of inlet air in any ten (10) successive samples. The technique of sampling shall be in accord with the method in Appendix B.

2.1.2 Bioclean Room Air: The concentration of viable particles in the room air shall not exceed an average of two (2) viable particles per cubic foot of bioclean room air for any ten (10) successive samples. The technique of sampling shall be in accord with the method in Appendix B.

2.1.3 Fallout Onto Surfaces: The degree of microbiological contamination accumulating on surfaces shall not exceed an average of 200 viable particles per square foot of surface. The technique of sampling shall be in accordance with the method in Appendix C.

2.1.4 Linens (Clothing and Packaging Materials): The degree of microbiological contamination on laundered clothing of non-sterilizable fabric shall not exceed three colonies per Rodac plate. Clothing of sterilizable fabrics shall be acceptable if no viable organisms are recovered from commercial spore strips contained in each clothing package during the sterilization procedure. The techniques of microbiological sampling shall be in accord with the method in Appendix D.

2.2 TEMPERATURE

The temperature within the bioclean room shall be 70° ± 10°F during the period occupied by workers. During periods when the room is unoccupied, the temperature may be raised to any level between 70° and 125°F.

2.3 RELATIVE HUMIDITY

The relative humidity of the bioclean room shall be 40% $^{+\ 5\%}_{-\ 0\%}$ at all temperatures, whether the room is occupied or unoccupied.

2.4 FLOOR SPACE

The bioclean room shall be constructed to provide a minimum of one hundred square feet of floor space per occupant.

2.5 MONITORING

2.5.1 General: Monitoring is for the purpose of measuring the level of microbiological loading on the spacecraft and detecting the occurrence of any accidental contamination. Microbiological assays of the products of bioclean rooms are not satisfactory by themselves as the basis for acceptance or rejection of the product. Such assays can be used only in conjunction with detailed inspection of the facility and operation. To facilitate the microbiological and visual monitoring of these groups, detailed records of all monitoring operations will be kept.

2.5.2 Visual Record: A 16-mm camera(s) shall be used to provide a continuous visual record of occurrences and operations in the bioclean room. The field of view of the camera(s) shall include the entire spacecraft assembly area. A maximum of two mirrors may be used to achieve this coverage providing the field of view of each mirror from the camera does not exceed ten (10) feet in width. The area shall be photographed, in color, at intervals of one frame per five (5) seconds during the entire time that flight hardware is in the area. A twenty-four (24) hour clock with sweep second hand and calendar date, readable from the projected image, shall be located in the field of view of the camera. Camera operation may be discontinued during periods when the bioclean room is not occupied by humans, and if the room is secured with time recording locks.

To review the projected image of the visual record, a viewing facility containing a projector, moviola, or any other device(s) approved by the Bioscience Program Division shall be available. The viewing facility shall be located in close proximity to, or in the same building as, the bioclean room.

To facilitate monitoring of simultaneous operations by one person, closed circuit television may be used to supplement the visual record.

2.5.3 Identification of Personnel: The identity and time of entrance and departure of every person entering and leaving the bioclean room shall be recorded. Recording may be done manually by a person assigned to control traffic. However, it is preferred that personnel recording should be done by an automatic system, such as an automatic recorder coupled to the door lock. Insertion of a magnetic card or key could simultaneously operate the lock and record time and identity of user. Alternate systems, approved by the Bioscience Programs Division, may be substituted.

Personnel entering the bioclean room shall wear an identifying number or symbol on his suit or cap. The identifying mark shall be large enough to be read in the projected image of the visual record.

2.5.4 Maintenance Operations: All maintenance operations such as changing of filters and cleaning in, or closely related to, the bioclean area shall be recorded with the following information:

Date
Time beginning
Time ending
Names of personnel
Description of work
Detailed location of work

2.5.5 Ventilation

2.5.5.1 Temperature: Temperature of the bioclean area shall be recorded continuously.

2.5.5.2 Relative Humidity: Relative humidity of the bioclean area shall be recorded continuously.

2.5.5.3 Pressure: Differential pressure, based on the pressure outside the bioclean area, shall be recorded continuously for the locker, dressing, and bioclean areas.

2.5.6 Microbioassays

2.5.6.1 Air: The microbiological quality of the ventilation inlet air and the room air shall be assayed not less than twice during each twenty-four hours that flight hardware occupies the bioclean area. The microbiological assay requirement for air would be waived for any twenty-four hour period that the bioclean area is completely unoccupied. Assay shall be performed in accord with the method in Appendix B.

2.5.6.2 Fallout: The degree of microbiological fallout onto surfaces within the bioclean area shall be assayed at least twice a week during each week that the area is occupied by spacecraft hardware. The assays shall be performed in accord with the method in Appendix C.

2.5.6.3 Linens: The degree of microbiological contamination on laundry provided for the use of bioclean room personnel shall be assayed in accord with the method in Appendix D.

2.5.7 Total Airborne Particles: The total airborne particulate matter shall be monitored continuously with an electromechanical light scattering device. Sampling results shall be in the form of a continuous display and printed digital record of the particle count with time of day. The minimum volume of air sampled for this purpose shall be 0.2 cubic foot per minute.

3. REQUIREMENTS - BIOCLEAN WORK AREAS

A hooded table or glove box may be used when parts of small size are to be manufactured or assembled under bioclean conditions. Products removed from the bioclean work area shall be enclosed in containers or wrappings meeting the standard for Linens, 2.1.4.

3.1 Portable Laminar Flow Clean Rooms: Portable clean rooms, consisting of a bank of filters that provide laminar flow of clean air over a work area enclosed by plastic walls, may be used when consistent with NASA policy. Design, construction, and operation shall be subject to approval by the Bioscience Programs Division. Characteristics of the environment within the portable clean room shall meet or exceed the standards described in Item 2 of this document.

3.2 Laminar Flow Work Benches: Tables covered with hoods and supplied with filtered ventilation air arranged to move from the work toward the operator may be used for work on assemblies of suitable size. Materials, design, and operation shall conform to the bioclean room requirements. Characteristics of the environment inside the hood shall meet or exceed the standards of Item 2 of this document.

3.3 Glove Boxes: Glove boxes shall be completely closed with static air, an inert gas, or a sterilant gas inside the chamber. Design, construction, and operation shall be subject to approval by the Bioscience Programs Division. A double door pass-through shall be used to provide a portal of entry of materials to, or from, the glove box. Rubber gloves, having the cuffs affixed externally to glove ports in the side of the box, shall be used to work inside the glove box. The internal environment of the glove box shall meet or exceed, where applicable, the standards of Item 2 of this document.

4. GLOSSARY OF TERMS

4.1 For the purpose of the interim requirements for bioclean facilities, the following definitions shall apply:

4.1.1 Bioclean room: An enclosed area employing positive control over the total (viable and non-viable) particulate matter in the air and on the surfaces therein, with temperature, humidity and pressure control as required. A bioclean room must maintain a particulate count as specified in one of the reference standards listed in Item 1.4 of this document. Furthermore, a bioclean room must maintain a viable particulate level at or below the limits stated in Item 2 (Requirements-Bioclean Rooms) of this document.

4.1.2 Bioclean work areas: A fixed, portable, or mobile working enclosure. Characteristics of the environment within the working enclosure shall meet or exceed the standards set for a bioclean room (Items 1.4 and 2 of this document).

4.1.3 Laminar airflow: Airflow having the entire body of air within a confined area moving with uniform velocity along parallel flow lines, with a minimum of eddies.

4.1.4 Microbiological aerosol: Microbiological contamination occurring as liquid or solid particles suspended in air.

4.1.5 Viable particle: Microbiological particulates may exist as single viable cells, or clumps of viable cells of one or more microbial species freely floating in air or settled onto surfaces. Viable particles may also be surrounded by a film of dried inorganic or organic matter, or they may be attached to dust or other inert particles.

4.1.6 Microbiological fallout: The deposition on surfaces of viable particles in a microbiological aerosol.

4.1.7 Bacterial spore: A refractile oval body formed within the cell of certain bacterial species. Bacterial spores are much more resistant than bacterial vegetative cells to injurious chemical and physical agents, including exposure to drying, heating and germicides.

4.1.8 Heat-shock: A procedure to destroy most, if not all, microbial vegetative cells in a spore-vegetative cell suspension. Heating the suspension to 80°C for 15 minutes will kill most, if not all, of the vegetative cells but should have little, if any, effect on the bacterial spores.

4.1.9 Spore strip: A known concentration of viable, dried bacterial spores contained on an oblong strip of filter paper.

APPENDIX A

The methods used to comply with these requirements are the burden of the responsible NASA Project, Center, and/or Contractor. The following methods of operation are recommended for use and are, in some cases, essential to achievement of the levels required.

A.1 Design and Contruction

A.1.1 Locker Room: A room should be provided equipped with benches, clothes lockers, toilet facilities, and hand washing facilities.

A.1.2 First Air Lock: A first air lock should be provided between the locker room and the dressing room. The doors should be interlocked so that only one door can be open at one time. Both doors should be gasketed to achieve an airtight seal when closed. An air shower equipped with an automatic twenty-second timer should be provided. The doors should be arranged to be locked while the air shower is in operation.

A.1.3 Dressing Room: A dressing room should be provided with construction features complying with the clean room specifications for materials and finishes. Clean clothing in kraft paper packages should be available in double door pass-through built into one wall. Alternatively the clothing may be brought into the room through such a pass-through and stored by clean room personnel in covered racks designed and used exclusively for this purpose. Wall hooks may be located on the contaminated area of this room to hold clean clothing while other garments are being donned. Small dressing stalls may be provided if they are designed to avoid the accumulation of dirt. A yellow and black "zebra" line, one-foot wide, should be positioned on the floor to mark the separation of the contaminated area of the dressing room from the clean portion. Personnel should never cross the "zebra" line without going through the required procedure. Rotary

stools, permanently affixed, should be placed in the center of the "zebra" line. Personnel should use these rotary stools to put on booties and rotate to the clean side of the line before putting the booties to the floor. Booties to be worn in the bioclean room should never touch the floor on the contaminated side of the "zebra" line. The door to the second air lock should be located in the clean area.

A.1.4 Second Air Lock: A second air lock should be provided between the dressing room and the bioclean room to prevent migration of airborne contamination. The doors should be interlocked, but no air shower should be used and no waiting period in the air lock need be required.

A.1.5 Floor Space: The total floor space should be kept as small as possible, consistent with the requirement for minimum space per occupant and the work to be done. All functions, equipment, and personnel which are not absolutely essential to the area should be excluded. As far as possible, large bioclean areas should be divided into small areas. One locker-dressing room complex can serve a number of bioclean rooms.

A.1.6 Shoe Cleaners: A minimum of two shoe cleaners should be provided. (1) One or more rotary brush-vacuum shoe cleaner designed for clean room use may be located adjacent to the entrance to the locker room. (2) A vibrating brush-vacuum mat shoe cleaner should be provided in the floor just in front of the locker room entrance door.

A.1.7 Entrances: There should be no entrance to the bioclean area other than through the locker-dressing room and air lock complex.

A.1.8 Exits: The service exit from the bioclean room should be through an air lock. The exit doors should be interlocked

and arranged to prevent egress to the bioclean area. The service exit should be arranged in such a manner that personnel cannot return to the bioclean area without again going through the locker room, dressing room and air locks. Emergency exits of a sealed, but "crash-panel", type may be provided.

A.1.9 Communications: Observation windows should be supplied in the walls of the bioclean room in locations such that work inside can be observed without need to enter the room. Intercommunication facilities may be provided between the control center (office), locker room, dressing room, and bioclean room. Wired multi-station intercommunication facilities should be used for general communication. Also, there should be a minimum of two intercommunication points, located beside observation windows on opposite (or adjacent) sides of the bioclean room, to provide communication through the wall of the room. At least one of these communication points should be located in close proximity to the equipment pass-through. Plastic diaphragm "speak-through" may be used for conversation at the pass-through or observation windows.

A.2 Ventilation

A.2.1 Air changes: A minimum of twenty (20) air changes per hour is necessary.

A.2.2 Air cleaning: The air entering the bioclean room should be cleaned by high-efficiency filtration (more than 95 percent), incineration, or any other method of proven reliability and efficiency approved by the Bioscience Programs Division. Electrostatic precipitation of viable particles is not recommended for bioclean areas.

A.2.3 Air Inlets: Air should be introduced into the bioclean room over as wide an area as possible. This may be done

by using the final filters as the ceiling or one wall, or a perforated ceiling may be used. If diffusers are used they should be located on centers not more than eight feet apart. Final filters should be located as near as possible to the point of air entry to the bioclean room. The aspirator type of diffuser should not be used in a bioclean room. Localized delivery of clean air over critical work areas may be used inside rooms meeting all other specifications.

A.2.4 Air Exhaust: Air should be exhausted from the bioclean room over the widest area possible, and near the floor level. A grating-type of floor with a plenum underneath the grating is ideal. The minimum practical exit for this purpose is the linear peripheral type of exhaust.

A.2.5 Air Pressure: The following pressure differentials, referenced to the pressure outside the locker room, should be minimal:

Locker room	+0.1 in. W.G.
Dressing room	+0.2 in. W.G.
Bioclean area	+0.4 in. W.G.

A.2.6 Air Recirculation: Up to 95 percent of the ventilation air supplied to the bioclean room may be recirculated; but all air, fresh or recirculated, should pass through the air cleaning system.

A.2.7 Pressure Reliability: The ventilation system should be designed and equipped to keep a positive pressure on the bioclean room, despite failure of any single motor, fan, or source of power.

A.2.8 Vacuum Cleaning: A central vacuum cleaning system capable of wet pickup should be provided for the bioclean

area and associated rooms. In specific cases, a portable vacuum cleaner may be used if its manner of operation is proven to be compatible with the microbiological requirements of this document. The design and construction of portable vacuum cleaners to be used should be approved by the Bioscience Programs Division.

A.3 Personnel Practices

A.3.1 Shoe Cleaning: All personnel entering the locker room should clean their street shoes thoroughly on the rotating brush shoe cleaner outside the locker room door. They should then pass over the vibrating brush-vacuum mat shoe cleaner as they enter the locker room. Only smooth-soled shoes should be permitted in the locker room.

A.3.2 Removal of Clothing: In the locker room all clothing and jewelry, such as rings, should be removed and stored in the individual lockers provided.

A.3.3 Transfer Gown (Optional): If desired, a freshly-laundered, lint-free, short-sleeved, coat-type garment may be provided and donned to be worn during washup and transfer to the dressing room.

A.3.4 Washing: Hands should be scrubbed thoroughly to a point above the wrists for a timed period of not less than three minutes. Only liquid soap containing hexachlorophene should be used. The liquid soap should be provided in a foot-operated dispenser located at each wash bowl. A clean, sterile surgical scrub brush should be used for each washing. Fingernails should be cleaned before and during washing.

Personnel should be encouraged to bathe after work or in the evening, using liquid hexachlorophene soap provided for that purpose by the employer. The employee should not bathe in the eight-hour period prior to entering the clean room.

A.3.5 Transfer to Dressing Room: After washing, personnel should enter the first air lock singly, or in groups sufficiently small to allow free air flow between individuals. The air shower should be operated for the prescribed time before the inner door to the dressing room is opened.

A.3.6 Dressing: Personnel may remove transfer gowns and place them in receptacles to be provided. From a newly opened package, they should don underwear, socks, coveralls and head covering. Clothing and packaging material should be placed in a receptacle provided. A package containing a pair of freshly cleaned booties should then be carried to the "zebra" line where the worker may seat himself on a stool. One bootie should be removed from the package and placed on the foot. Care must be taken to insure that the bootie does not come in contact with the floor on the contaminated side of the line. The worker can partly rotate the stool, placing the bootied foot on the "clean" side of the line, and repeat the process. Shoes and packaging material should be placed in a receptacle provided.

A.3.7 Transfer to Bioclean Room: Personnel should inspect each other for correctness of dress and then enter the second air lock. They may open the second door and proceed into the bioclean room as soon as the first door is closed and secured.

A.3.8 Departure from Bioclean Room: Personnel leaving the bioclean room shall use the air lock designated for

egress. Emergency exits should only be used for emergency situations. Return to the bioclean area must be through the locker room-dressing room route with repetition of the required procedure.

A.3.9 Gloves: Sterile surgical rubber gloves shall be worn to handle cleaned spacecraft parts. Disposable, sterile plastic gloves may be substituted for the sterile surgical rubber gloves. Sterile clean room fabric gloves may also be used, but following use of these gloves the area of the part contacted by the gloved hand must be wiped by a clean, sterile cloth moistened with a germicide approved by the Bioscience Programs Division. Alternatively, cleaned parts may be touched and handled with sterile instruments.

A.3.10 Tool Use: Bioclean room tools should be kept clean and, preferably under stile conditions. After use, tools should be heat sterilized. The ultrasonic cleaning and/or heat sterilization procedure should be applied to all tools that are dropped during use, or to tools that are placed on contaminated surfaces.

A.3.11 Traffic Control: A positive means of traffic control shall be in effect at all times. A control system may consist of a guard stationed at the dressing room door to admit only authorized persons. Alternatively, an automated system may be used.

A.3.12 Visitors: Visitors should not be allowed to enter the area when flight hardware is present in the bioclean room. If necessary, visitors may view the work through the observation windows and use the intercommunications system for conversation. Specific personnel, who have proven a need to enter the bioclean room while flight hardware is housed therein, may be permitted entrance to the bioclean area. Such personnel must go through

the same pre-entry procedures as bioclean room personnel and must be accompanied, at all times, by an authorized supervisor.

A.3.13 Personnel Health: No individual, regardless of need, should enter the bioclean area if he has; a cold and/or a fever, readily noticeable dandruff, diarrhea, or any other condition that, in the opinion of the attendant medical officer, would contribute an increased level of intramural microbiological contamination.

A.3.14 Eating and Smoking: Eating, expectorating, or the use of any form of tobacco shall be prohibited within the bioclean area.

A.4 Maintenace

A.4.1 Cleaning of Ventilation System: Periods when flight hardware is removed from the bioclean area should be used to clean the ventilation system and to change filters and other elements. Plenum and duct walls may be cleaned with a vacuum cleaner if there is visible soil. Following change of filters, the bioclean area should be thoroughly cleaned. The ventilation system should be operated for a minimum of 48 hours, followed by microbiological assays to prove the filtering efficiency of the new filters and/or elements as installed.

A.4.2 Vacuum Cleaning of Floors, Walls and Fixtures:
A.4.2.1 Horizontal Surfaces: Horizontal, upward facing surfaces should be vacuumed daily or as often as required to maintain the specified contamination levels.

A.4.3 Vertical Surfaces: Vertical surfaces and downward facing horizontal surfaces (ceilings, under table tops) may be vacuumed weekly, or as often as required to maintain the specified contamination levels.

A.4.4 Wiping of Surfaces: Walls, floors, ceilings, and working surfaces may be washed with synthetic sponges soaked in 90 percent denatured ethyl alcohol, Dow Fax 9N9 or equivalent detergent-germicide solution at least once a week.

A.4.5 Inspection: Operating and supervisory personnel should survey the area daily for any unnecessary accumulation of parts, materials, or equipment that could trap or be possible sources of contamination. Visible dirt or dust should be removed immediately.

APPENDIX B

Method of Sampling Microbiological Contamination in Ventilation Air

B.1 Equipment

B.1.1 Air Sampling Device and Supporting Equipment: One (or more) slit-type solid medium impaction sample with coupled tapered tube flow meter and a one-hour clock to rotate plate beneath the slit (1.0 revolution per hour).

B.1.2 Vacuum Pump(s): One (or more) vacuum pump capable of drawing 1.2+ cubic feet of air per minute at one-half atmosphere. Pump to be located outside the bioclean room.

B.1.3 Isokinetic Probe(s): One (or more) isokinetic probe to match the velocity of air flow in the duct to be sampled.

B.1.4 Culture Medium: Trypticase soy agar.

B.1.5 Miscellaneous: Petri dishes to fit the sampler, rubber and/or Tygon tubing, stand or table, and clamps to hold the sampler, tubing, etc.

B.1.6 Sampling Frequency:

B.2 Sampling Procedure

B.2.1 Duct Air: An isokinetic probe should be used to collect a representative sample from a ducted air stream. The probe should have thin walls, as large an I.D. as possible, (minimum 0.25 in.), head directly upstream, and a sharp edged orifice. The inside diameter of the orifice shall be such that, at the sampling rate of one cubic foot per minute, the velocity of the air entering the orifice shall be within plus or minus ten percent of the velocity of the air stream being sampled. The probe may have a

maximum of one 90-degree bend through a radius not less than eight inches. The probe can be inserted through a hole in the duct and be held in place by a plate and self-tapping screws. Cover plates with screw holes matching the probe plate can be used to cover the sampling hole when not in use. The outer end of the probe should be connected to the entry tube of the sampler by Tygon tubing. This connecting Tygon tube should be kept as short as possible. The connecting Tygon tube should be kept as short as possible. The connecting Tygon tube should contain a minimum number of bends. Where bends are unavoidable, they should have the largest possible radius.

The Tygon tube can be adapted to the entry tube of the sampler by means of a short section of metal tube passed through a rubber stopper inserted into the open end of the sampler inlet slit tube. The isokinetic probe, connecting tubing and adapter should be sterilized by autoclaving between each series of samples on any one duct.

B.2.2 Room Air: To sample room air, the sampler should be located near the center of the room, with the inlet slit in a horizontal position. The inlet slit should be four (4.0) to five (5.0) feet above the floor. Air flow rate through the sample should be one (1.0) cubic foot per minute (cfm). Duration of the sample period should be sixty (60.0) minutes.

B.3 Incubation and Analysis

B.3.1 Incubation conditions: Upon completion of the sampling period(s) the plate(s) will be covered, removed from the sampler(s), and incubated at 32°C for 72 hours.

B.3.2 Analysis: Following incubation, the resultant colonies will be counted. The number of viable particles per

cubic foot of air can be determined by dividing the colony count per plate by 60.

B.3.3 Controls: Appropriate sterility checks should be made on each prepared lot of plates containing Trypticase soy agar.

B.3.4 Reference: Wolf, H. W1, et al, "Sampling Microbiological Aerosols", Public Health Monograph No. 60, Government Printing Office, Washington, D. C. (1959).

APPENDIX C

Method of Sampling Viable Particle Fallout on Surfaces

C.1 Equipment

C.1.1 Fallout Sampling (settling) Strips: Sterile, 1.0 by 2.0 by approximately 0.06 in. (ca. 16.0 gauge) strips of stainless steel should be used to sample the viable particle contamination that accumulates on surfaces within the bioclean room.

C.1.2 Carrier for Fallout Sampling Strips: Any conveniently sized shallow metal pan. Edges of the pan should not exceed 0.5 in. in height.

C.1.3 Culture Media

C.1.3.1 Rinse fluid: Sterile, one (1.0) percent peptone water.

C.1.3.2 Plating agar for rinse fluid: Trypticase soy agar.

C.1.4 Glassware: Sterile glassware as required.

C.2 Sampling Procedure

C.2.1 Preparation of Fallout Sampling Strips for Use:

C.2.1.1 Washing:

a. Wash in hot tap water containing detergent

b. Rinse with hot distilled water

c. Rinse with isopropyl alcohol

d. Rinse with ether

e. Drain dry

C.2.1.2 Sterilization: Cleaned strips and carrier tray sterilized by dry heat, 180°C for 90 minutes.

C.2.2 Exposure of the Sterile Fallout Sampling Strips: Using sterile forceps, the sterile strips may be laid onto the bottom of the sterile carrier tray as a single layer of sterile strips. The distance between strips in the layer should be such that individual strips may be removed singly without disturbing adjoining strips. The sterile carrier tray and sterile strips should be placed in the bioclean room in a location where they will not be disturbed. However, the strips should have equivalent exposure to the air circulating in the bioclean room as prevails at bench top level and/or at other assembly areas. The number of exposure sites shall be dependent upon the size of the bioclean room and/or on the type of hardware being assembled therein.

C.2.3 Sampling Frequency: Six (6.0) exposed strips shall be removed from each carrier tray at least twice weekly (Monday and Friday).

C.3 Microbiological Assay

C.3.1 Removal of Viable Particles from Fallout Sampling Strips:

At the required intervals, the six test strips shall be removed singly from the carrier tray with sterile forceps. Care should be taken to avoid sliding the test strip on the carrier tray or to avoid disturbing adjacent strips. Each strip shall be placed in a sterile screw cap bottle containing fifty (50.0) ml of sterile one (1.0) percent peptone water. The sample bottle should have a wide mouth to facilitate insertion of the test strip. The shape and size of the sample bottle should be such that the long side of the strip remains approximately parallel with the sides of the bottle. The rinse fluid should cover approximately 50 to 70 percent of the inserted strip. The shape and size of the sample bottle

should remain constrant. The sample bottles, containing the inserted test strips and rinse fluid, shall be mechanically shaken for five (5.0) minutes.

C.3.2 Culturing of Viable Particles Removed from the Fallout Sampling Strips:

C.3.2.1 Incubation: Four (4) of the six (6) test strips in each microbiological assay should be incubated aerobically at 32°C for 72 hours. The remaining two (2) test strips should be incubated anaerobically at 32°C for 72 hours.

C.3.2.2 Treatment of Rinse Fluid after Test Strip Shaking: After the five (5.0 minute shaking period, the rinse fluid shall be aseptically removed from sample bottle-test strip combination and divided into two approximately equal portions.

C.3.2.3 Plating of Rinse Fluid Portions:

C.3.2.3.1 None-heat Shocked Portion: The volume of rinse fluid plated should be dependent upon the concentration of viable particles in suspension. One (1.0), five (5.0) or approximately twenty-five (25.0) ml aliquots may be plated in the Trypticase soy agar medium. The completed pour plate(s) should be incubated aerobically or anaerobically as defined in C.3.2.1 above.

C.3.2.3.2 Heat-shocked Portion: The remaining portion of rinse fluid from each test strip should be heat-shocked (80°C for 15.0 minutes) to determine the presence of microbial spores. After heat-shocking, the

volume of rinse fluid plated and the incubation conditions shall be as C.3.2.3.1 above.

C.4 Sampling Frequency: Six (6) test strips from each exposure site. Strips to be collected and assayed twice per week (Monday and Friday.

C.5 Control: Sterile, covered fallout sampling strips should be exposed and assayed during each test strip assay. Control plates should remain sterile.

C.6 Reporting: The number of viable particles will be expressed as contamination per square inch.

C.7 Reference:

1. Hoffman, R. K. et al. Protection Branch Test Reports, Fort Detrick, Frederick, Maryland.

2. McDade, J. J. "The Microbiological Profile of Clean Rooms". Jet Propulsion Laboratory Space Program Summary. In Press.

APPENDIX D

Method of Sampling Microbiological Contamination on Linens

D.1 Equipment

D.1.1 Rodac plate: Rodac plates containing 15.5 ± 0.1 ml culture medium. Plates pretested to assure sterility of unit.

D.1.2 Culture medium: Trypticase soy agar.

D.1.3 Spore strips: Any commercially available product approved by the Bioscience Programs Division.

D.1.4 Glassware: As required.

D.2 Sampling Procedures:

D.2.1 Non-sterilizable Fabric

D.2.1.1 Rodac Plate Method: Clean room clothing should be tailored to contain several removable patches of the same material placed externally at fixed sites (e.g., shoulders, armpits, on the front at bench level, and at knee length). The patches may be attached to the clean room clothing immediately prior to laundering. The laundered clothing may be sampled by removing the patch and pressing the agar surface of a Rodac plate onto the inner side of the removed patch. The Rodac plate may then be covered and incubated aerobically at 32°C for 72 hours. The minimum sample shall consist of three (3.0) separate Rodac plate replicates on a single surface.

D.2.1.2 Sample Frequency: At least one uniform in each laundered package should be sampled. When a given laundry procedure consistently produces clothing of acceptable microbiological cleanliness, the sampling frequency may be reduced to random spot checking of laundered packages.

D.2.1.3 Reference:

1. Bond, R. G., et al, "Development of a Method for Microbial Sampling of Surfaces with Special Reference to Reliability." Report on Public Health Service Contract PH-86-62-182, University of Minnesota, Minneapolis (1963).

2. Hall, L. B. and Hartnett, M. J., "Measurement of Bacterial Contamination on Surfaces in Hospitals". In Press.

D.2.2 Sterilizable Fabric

D.2.2.1 Spore Strip Sterility Indicator: Sterilizable fabric for clean room clothing was either not available or its use considered impractical at the time this document was prepared. Should garments of sterilizable fabric become practical in the future, it may be possible to insert a commercially available spore strip(s) into each bundle of clean clothing to be sterilized. The sterilized bundle shall be considered microbiologically acceptable if viable spores are not recovered when the spore strips in the treated package are cultured according to the procedure specified by the manufacturer of the strips.